

United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

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Title: Isolation and Identification of <i>Escherichia coli</i> O157 by Immunomagnetic Separation (IMS) and Cultural Methods		
Revision: 03	Replaces: 01/01/06	Effective: 05/01/07

1. Purpose

This Standard Operating Procedure (SOP) is used after completion of SOP MDP-MTH-05 yielding a positive result for *Escherichia coli* (*E. coli*) O157:H7. This document provides standard procedures for capturing *E. coli* O157 cells by immunomagnetic separation, subculturing to various selective agar media, and identifying isolates by VITEK[®]. Isolates can be further identified by serotyping.

2. Scope

This SOP shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory. IMS is used as one of the methods in the isolation of *E. coli* O157.

3. Principle

The immunomagnetic separation (IMS) method offers a means to concentrate target bacteria from mixed cultures by physical separation based on an antigen-antibody reaction. Selective capture (concentration) of bacterial cells is achieved by antibodies, specific to the cell surface antigens of the target strain, immobilized on superparamagnetic polystyrene micro-beads. After washing the beads to remove non-target organisms, the magnetized beads coated with target bacteria are recovered and processed for isolation using various selective agar media and identification by cultural methods and serotyping.

4. Outline of Procedures

Equipment and Materials	6.1
Media and Reagents	6.2
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5. References

- 5.1. Enrichment of *E. coli* O157, Product information for Dynabeads[®] anti-*E. coli* O157 or
[http://www.dynalbiotech.com/kunder/dynal/DynalMMA401.nsf/lupgraphics/710_04_Dynabeads_antiEcoli_O157.pdf/\\$file/710_04_Dynabeads_antiEcoli_O157.pdf](http://www.dynalbiotech.com/kunder/dynal/DynalMMA401.nsf/lupgraphics/710_04_Dynabeads_antiEcoli_O157.pdf/$file/710_04_Dynabeads_antiEcoli_O157.pdf) (last accessed 11/10/05)
- 5.2. USDA/FSIS Microbiology Laboratory Guidebook Chapter 5, Revision 2
Detection, isolation, and identification of *Escherichia coli* O157:H7 and O157:NM (non motile) from meat products,
<http://www.fsis.usda.gov/ophs/microlab/mlg5.03.pdf> (last accessed 01-09-07)
- 5.3. Peter Feng and Stephen Weagant. 2002. Diarrheagenic *Escherichia coli*.
Bacteriological Analytical Manual Online. Section: Isolation and confirmation of *E. coli* O157:H7. <http://www.cfsan.fda.gov/~ebam/bam-4a.html> (last accessed 01-09-07)
- 5.4. SOP MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX[®] PCR
- 5.5. SOP MDP-QA-03, Quality Assurance (QA) Controls
- 5.6. SOP MDP-SHIP-03, Procedures for Packaging, Shipping, and Archiving Microbiological Cultures
- 5.7. SOP MDP-DATA-01, Microbiological Record Keeping and Results Reporting

6. Specific Procedures

- 6.1 Equipment and Materials
 - 6.1.1 Additional materials needed to perform procedure as listed in the Dynabeads[®] anti-*E. coli* O157 product instruction or suggested by the manufacturer
 - 6.1.2 VITEK[®] (bioMerieux, Inc.) System and Users Manual
 - 6.1.3 VITEK Cards: GNI+ card (gram-negative plus) V1316
 - 6.1.4 Incubator 35 ± 2°C
 - 6.1.5 Incubator 42 ± 2°C.

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6.2 Media and Reagents

- 6.2.1 Dynabeads[®] anti-E. coli O157 (DynaL Biotech)
- 6.2.2 Wash buffer (PBS Tween): 0.15M NaCl, 0.01M Sodium-Phosphate buffer, pH 7.4, with 0.05% Tween-20; autoclaved at 121°C for 15 minutes
- 6.2.3 Modified EC broth (mEC) with Novobiocin (mEC+n). Refer to FSIS-MLG; <http://www.fsis.usda.gov/ophs/Microlab/Appendix1.02.pdf> for the broth composition. This broth can be homemade or purchased commercially. Follow the supplier's directions for reconstitution, stability and storage of novobiocin and its addition to the broth.
- 6.2.4 CHROMagar[™] O157, DRG International (or demonstrated equivalent) with supplements (e.g. tellurite, cefixime, cefsulodin etc.)
- 6.2.5 Tellurite-Cefixime Sorbitol MacConkey (TCSMAC) agar
- 6.2.6 CHROMagar[™] *E. coli* from DRG International (or demonstrated equivalent)
- 6.2.7 L-EMB agar
- 6.2.8 MacConkey agar (MA)
- 6.2.9 Blood agar plates (BA)
- 6.2.10 Commercially available agglutination test kits for somatic O and flagellar H antisera for *E. coli* O157:H7 (Pro-Lab Diagnostics or Remel Microbiology Products or demonstrated equivalent)

6.3 Controls: (Specific strains are listed in SOP MDP-QA-03).

- 6.3.1 Carry all cultural controls from SOP MDP-MTH-05 through this entire procedure. Refer to SOP MDP-LABOP-02 for control setup.
- 6.3.2 If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03.

6.4 Safety

E. coli O157:H7 is a human pathogen and is known to cause disease with a low infectious dose. The laboratory personnel must follow CDC guidelines for working with Class II pathogens. Use of lab coats, gloves, and eye protection is mandatory. A Class II biosafety laminar flow hood (cabinet) is recommended.

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6.5 Isolation

- 6.5.1 Use the BAX[®] *E. coli* O157:H7-positive UPBt preenriched overnight culture identified using SOP MDP-MTH-05.
- 6.5.2 Streak and/or plate (0.1 mL or can be diluted) the BAX[®]-positive culture on the selective agar plates, CHROMagar O157 and TCSMAC, for isolation. Incubate at 35 ± 2°C for 18-24 hours. Incubate a second set of selective agar plates at 42 ± 2°C for 18-24 hours.
- 6.5.3 Transfer, in duplicate, 25 mL BAX[®]-positive culture to 225 mL sterile mEC+n (normally a 10 fold dilution is recommended). Incubate at 42 ± 2°C for 18-24 hours. Incubate the duplicate culture at 35 ± 2°C for 18-24 hours.
- 6.5.4 Plate or streak each mEC+n enriched culture on selective agar plates CHROMagar O157 and TCSMAC, for isolation. Incubate plates at 35 ± 2°C for 18-24 hours.
- 6.5.5 Plate or streak each mEC+n enriched culture on selective agar plates CHROMagar O157 and TCSMAC, for isolation. Incubate plates at 42 ± 2°C for 18-24 hours.

Note: Use of several selective agar plates, both in type and number, will improve the chance of finding the target isolates on plates. Use two types of selective agar plates, CHROMagar O157 and TCSMAC for screening at two different temperatures. BAX[®]-PCR on mEC-enriched cultures can be performed to verify the presence of *E. coli* O157:H7.

- 6.5.6 Perform IMS on the BAX[®]-positive samples, both UPBt and mEC+n-enriched cultures, following the manufacturer's instructions or validated IMS procedure. Process three to five 1 mL BAX[®]-positive samples along with one each of positive and negative control samples.
- 6.5.7 Dispense the concentrated samples to several plates of CHROMagar[™] O157 and TCSMAC. Incubate at 35 ± 2°C for 18-24 hours.

Note: If more concentrated sample is left, streak or plate a second set of selective agar plates and incubate at 42 ± 2°C for 18-24 hours.

- 6.5.8 Observe plates for typical *E. coli* O157 colonies.

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Typical colony characteristics of <i>E. coli</i> O157:H7		
Medium/Test	Colony Characteristics	Phenotype
L-EMB w/ lactose	Greenish with dark center	Lactose positive
MacConkey w/ lactose	Red	Lactose positive
CHROMagar™ <i>E. coli</i>	White	MUG (GUD) negative
CHROMagar™ O157	Mauve	Proprietary physiological test for O157
TCSMAC	Colorless with gray center	Sorbitol negative

6.5.9 Pick a minimum of 5-10 typical colonies (if available) on the selective agar plates used for isolation. Restreak them on CHROMagar™ O157, TCSMAC, CHROMagar™ *E. coli*, and L-EMB or MacConkey plates. Incubate at $35 \pm 2^{\circ}\text{C}$ for 18-24 hours. Check for purity and colony characteristics. Select typical colonies and streak them on BA plates.

6.6 Identification of *E. coli* O157:H7

6.6.1 Select 3-5 typical colonies from BA and identify using VITEK® or another official standard method of identification.

6.6.2 If biochemical assay results are consistent with the *E. coli* O157 profile, proceed with serology.

6.6.3 Select 3-5 presumptive positive isolates and perform serology per manufacturer's directions and internal laboratory procedures. Use small amounts of growth from single colonies for the agglutination tests.

6.6.4 Select one typical colony that has been confirmed by BAX® and serotyping as *E. coli* O157:H7 for archiving and shipping.

6.7 Reporting and Shipping

6.7.1 A final positive result is defined as a culture that is biochemically identified as *E. coli* and possible *E. coli* O157:H7 and serologically confirmed as *E. coli* O157:H7.

6.7.2 Immediately following completion of biochemical and serological tests, submit final results on SOP MDP-DATA-01 Attachment 01, "Preliminary/Final Results Notification Form".

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6.7.3 Report results according SOP MDP-DATA-01.

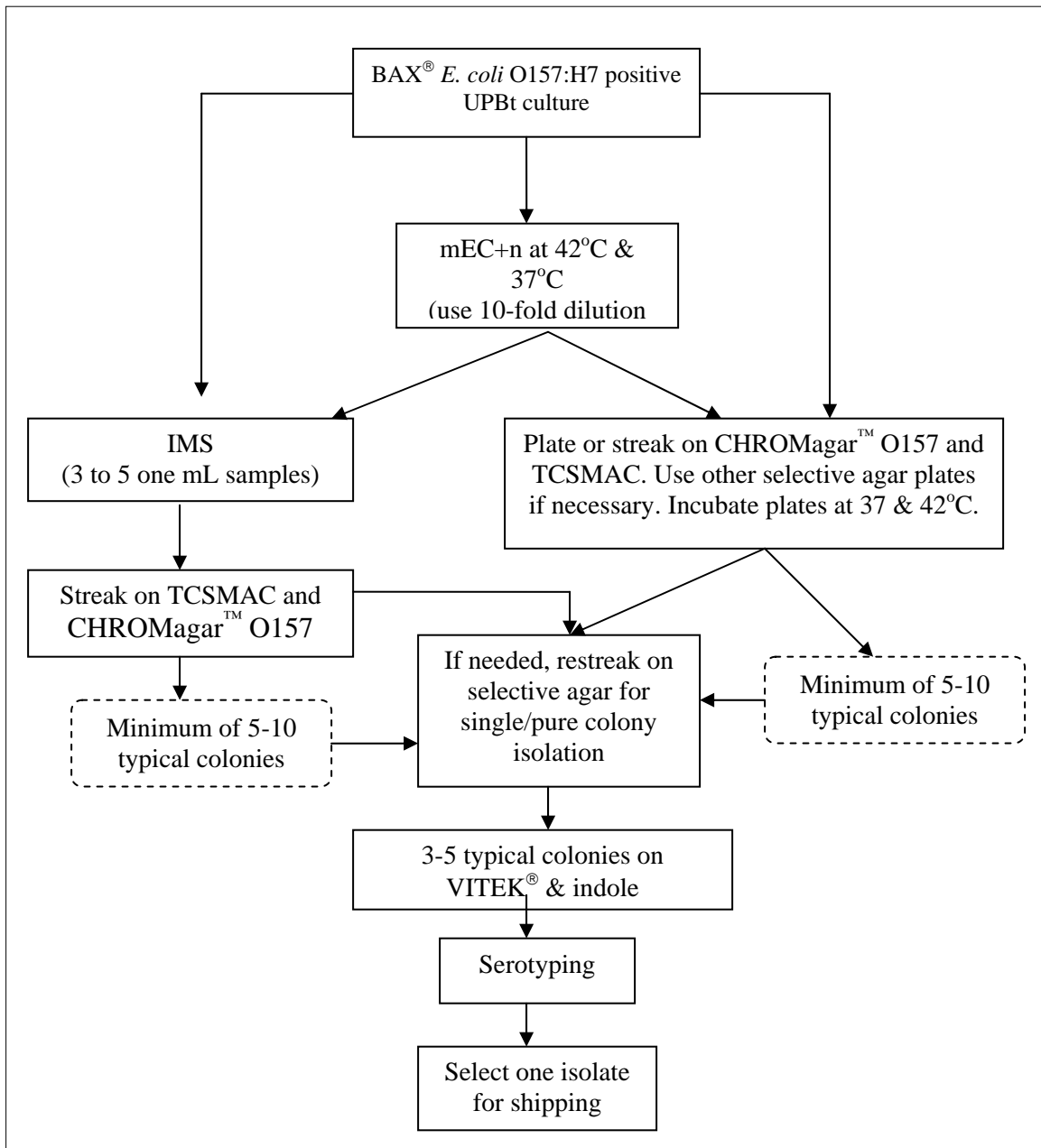
6.7.4 Refer to SOP MDP-SHIP-03 for preparation of cultures for shipment.

Disclaimer: Reference to brand names (kits, equipment, media, reagents, etc.) does not constitute endorsement by this agency.

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***E. coli* O157:H7 Isolation**



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Revision 03 April 2007 Monitoring Programs Office

- Added mEC+n broth for enriching BAX[®] PCR *E. coli* O157:H7 positive cultures at 42°C and 37°C.
- Added incubation of selective agar plates at two different temperatures, 37 and 42°C.

Revision 02 January 2006 Monitoring Programs Office

- Expanded isolation and identification steps
- Introduced serotyping
- Changed the requirement for IMS processing of a positive sample from one 1.0 mL sample to three to five 1.0 mL samples.
- Changed IMS to one of the methods in the isolation process

Revision 01 January 2005 Monitoring Programs Office

- Changed controls
 - Modified to refer to manufacturer's instructions for the IMS protocol
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